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SPERMATOGENESIS IN DIOON EDULE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 125

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(WITH PLATES XV–XVIII AND THREE FIGURES)

In March, 1904, and again in September, 1906, the writer visited Mexico for the purpose of studying the Mexican cycads and collecting material for morphological work. The field study was greatly facilitated by the active cooperation of Governor TEODORO A. DEHESA, whose active interest in education has done so much to raise the educational standard of the State of Vera Cruz. I am also deeply indebted to Mr. ALEXANDER M. GAW, of the State Bureau of Information, Xalapa, Mexico. Mr. GAW not only supervised the collection of material and forwarded it to me, but on several occasions visited localities where *Dioon edule* grows and sent me valuable field notes with the collections. The material was collected at Chavarrillo, Mexico, the locality which furnished material for my account of the ovule and female gametophyte of *Dioon edule.*

THE STAMINATE CONE

In March the ovulate cones which were pollinated the previous September have reached their mature size, but the staminate cones from which they were pollinated have disintegrated. Both the ovulate and the staminate cones which are to appear a few months later are still hidden in buds which cannot be distinguished from leaf buds.

Until they reach a length of about 10^{cm} the staminate cones are completely hidden by large bud scales which are fleshy and very hairy, so that the whole structure looks like a moderate-sized ovulate cone. The surface of the cone at this time is densely covered with whitish hairs, which become darker when the cone emerges from the protecting scales.

In September the staminate cones reach their mature size and shed their pollen. Just before the pollen is shed, the cone is firm and erect (*fig. 1*) and measures 10 to 20^{cm} in length and 7 to 11^{cm} in diameter. At the time of shedding the pollen, the axis of the cone elongates considerably and becomes so weak that it bends over until its tip rests upon the leaves of the crown. Insects are very numerous in nearly all the mature staminate cones, but none were found on the ovulate cones, and although the insects were throughly dusted with pollen, there was nothing further to indicate that any pollination was being effected through their agency.

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[1] Investigation prosecuted with the aid of a grant from the Botanical Society of America.

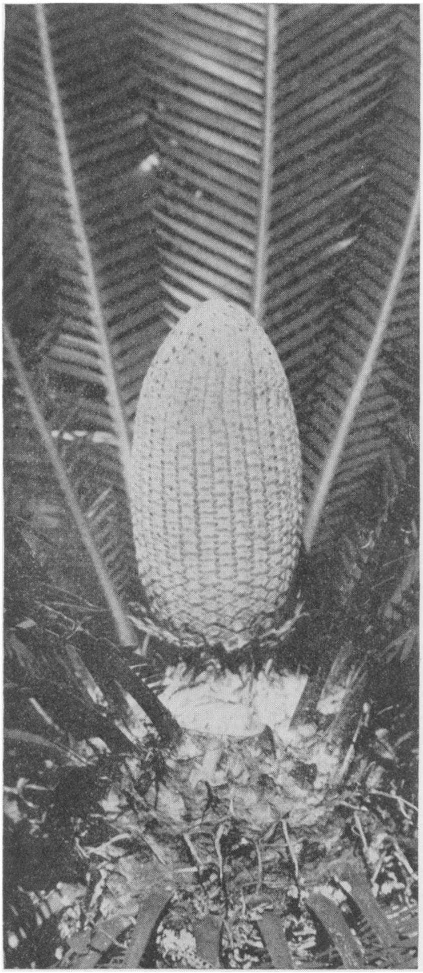

FIG. 1.—Upper portion of plant of *Dioon edule* with staminate cone. Photographed at Chavarrillo, Mexico, September, 1906. One-third natural size.

MICROSPORANGIA

The staminate sporophylls are long and wedge-shaped and end in a single sharp point which curves upward (*fig. 2*). The outer, exposed, abaxial faces of the sporophylls are densely hairy, but the upper faces are quite smooth. In the sporophylls of the upper and lower portions of the cone, a sterile line divides the sporangia into two groups (*fig. 2*), while in the median portions the sporangia cover the entire undersurface. The sporophylls are so crowded in the cone that the sporangia on the under side of a sporophyll make a distinct impression upon the upper side of the sporophyll immediately below it.

The number of sporangia on the larger sporophylls varies from about 100 to 300; but the smaller sporophylls bear fewer, and the sporophylls at the apex and base of the cone may bear only a few sporangia, or even none at all.

The sporangia are grouped in definite sori, usually of four or five sporangia, as shown in *fig. 2*. At the stage shown in this figure the line of dehiscence is quite obvious. As the sporangia dehisce, the grouping into sori becomes less obvious. The pollen does not escape immediately, but for a short time is held together in a spherical mass by the scanty remains of the hypodermal wall layers. A hand lens shows that each sorus is surrounded by hairs which grow out from the peripheral portion of the base of the sorus. Many hairs also grow out from the peripheral portions of the sporangia, but there are none in the interior of the sorus, either upon the sporangia or upon the sporophyll (*fig. 4*).

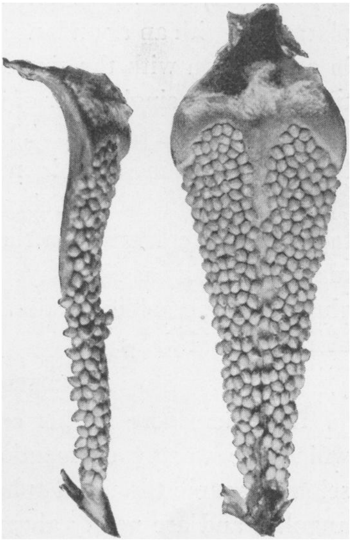

FIG. 2.—Photographs of two microsporophylls of *Dioon edule*. × $\frac{4}{8}$.

The sporangia are either sessile or have short massive stalks. The stalks and even the lower portions of the sporangia of a sorus may be somewhat united, but there is not so much crowding or so much union as shown by Miss SMITH (**8**) for Zamia and Ceratozamia. The dehiscence, in surface view and in section, is about as figured by Miss SMITH (**8**) for Zamia and Encephalartos.

The general appearance of a sporophyll in transverse section, with its mucilage ducts, vascular bundles, and sori, is shown in *fig. 3*. A more detailed view of two of the sporangia of a sorus is seen in *fig. 4*, which shows the usual amount of union at the base of the sorus. The hairs, especially those which come from the sporangia, are often so numerous and crowded that in transverse section they look more like a parenchyma tissue of angular cells than like sections of tubular hairs.

The hairs do not branch and seldom consist of more than two cells, which are sometimes empty and sometimes filled with a deeply staining substance like that in the epidermal cells of the sporangium.

The wall of the sporangium is composed of three distinct regions, the epidermis, the tapetum, and the intervening wall layers. The epidermis, which is thicker in the upper half of the sporangium than in the lower, consists of thick-walled cells with rather scanty protoplasm, but with an abundance of suberin and tannin. The tapetum, in comparison with the size of the sporangium, is very insignificant, consisting of a single layer of small cells, with occasional patches two cells in thickness (*fig. 5*). The portion of the sporangium wall facing the center of the sorus is noticeably thinner than the wall of the opposite portions, there being four or five layers of cells between the tapetum and epidermis in the former case, while in the latter there may be as many as eight layers (*fig. 4*). The structure of the sporangium from the epidermis down to the sporogenous tissue is shown in more detail in *fig. 5*.

MICROSPORE MOTHER CELLS

The microspore mother cells of Dioon present some peculiarities which are worthy of mention. Upon becoming dissociated, they seldom assume the usual spherical contour, but remain more or less angular, and are nearly always elongated. The chromatin is abundant, but not always well defined, and it is not unusual for the entire nucleus to stain a dense homogeneous black with iron alum hematoxylin, as if chromatin had gone into solution in the nuclear sap. In such cases, there are in the cytoplasm irregular masses of similarly staining material which take the spherical form and begin to resemble nucleoli as the homogeneous staining of the nucleus ceases and the chromatin becomes definitely outlined. When these spherical bodies were first observed, an effort was made to connect them with the blepharoplast, but it was easily determined that they were formed by the rounding-off of the irregular masses, and that they are never surrounded by radiations. They vary in number and position, but this may be true of young blepharoplasts. While the origin of the masses was not determined absolutely, there is little doubt that they represent a portion of the deeply staining material which has passed from the interior of the nucleus into the cytoplasm.

The behavior of the chromatin during the two mitoses by which four spores are formed from the mother cell will be described at some future time; at present I merely note that the number of chromosomes in both mitoses is 12. A hasty examination might lead one to suspect that the number is much larger, since it is not difficult to find prophases of the first mitosis, just before the disappearance of the nuclear membrane, showing any number of chromosomes from 12 to 24. But when there are more than 12, some are always about half the full size. Any number beyond 12 is due to the early separation of the two parts of the chromosome, which, in most cases, are separated only after the chromosomes have become arranged in the equatorial plate. Even in prophase, there are occasional indications of the second splitting which is to be completed at the metaphase of the second mitosis.

These mitoses are not simultaneous throughout the whole sporangium, but begin at the periphery and proceed toward the center, so that there may be a zone of dividing cells surrounding mother cells which are still in the resting condition.

POLLEN BEFORE SHEDDING

The young pollen grains are not quite spherical, there being a flattened portion which might be called the base of the grain (*fig. 6*). The exine is thickest in this basal region, while at the opposite end of the grain where the pollen tube is to emerge, it is very thin. The intine is thinnest in the basal region where it is in contact with the thickest portion of the exine. On the sides of the spore, the intine is very thick, often thicker than the exine. There is no trace of a third spore coat, as described by FERGUSON (4) for Pinus.

The microspore germinates while still in the sporangium. A single persistent prothallial cell is formed, lenticular in shape and closely applied to the base of the spore. WEBBER (2) described two prothallial cells in Zamia, the first formed being evanescent and the second persistent, but a reexamination by Miss GRACE SMITH (8) showed only one. We find only one prothallial cell in Dioon, Zamia, and Encephalartos, and it is persistent. In later stages, after the pollen tube has begun to form, it would be easy to misinterpret, for the line of union of the stalk cell with the persistent prothallial cell often gives the impression of a small prothallial cell beneath the large persistent one (*figs. 10–12*). The illusion is emphasized by the fact that deeply staining granules simulating a broken-down nucleus are sometimes found at the base of the prothallial cell (*fig. 15*). It seems probable that a misinterpretation of this sort was responsible for the description of an evanescent prothallial cell.

The nucleus in the main body of the grain now divides again, the mitosis resulting in the formation of a tube cell and a cell which resembles the prothallial cell and becomes so closely applied to it that the two look as if they had arisen by the division of the prothallial cell (*figs. 7–9*). This cell, so closely associated with the prothallial cell, has been called the generative cell. It soon divides, giving rise to the stalk and body cells (*fig. 10*). The tube nucleus, even before the formation of the tube, increases greatly in size, and the cell which is to form the pollen tube becomes filled with large starch grains. Late in September the pollen is shed in this three-celled condition.

The output of spores can be estimated with reasonable accuracy by the formula $\frac{4}{3}\pi R^3 =$ the number of spores in a sporangium. To apply the formula, it is necessary only to count the number of spores in a line from the center of the sporangium to the tapetum, substitute this number for R, and then make the calculation. Of course, this assumes that the mass of spores is spherical and that all spores develop, both of which assumptions are more or less incorrect, but the error is easily less than the variation in the output of individual sporangia of average size. In a few cases, spores were actually counted in a series of sections and the results were practically identical with the estimates by the formula. In the larger sporangia there are about 20 spores in a radius, and consequently the output is about 33,507 spores. The largest sporangia, with a radius of 22 spores, would have an output of 44,600 spores. In some of the smaller sporangia, which nevertheless produce good spores, the output may fall as low as 8000 spores. About 30,000 spores may be regarded as a typical output for the average sporangium of *Dioon edule*.

Late in September or early in October, when the pollen is shed, a large pollination drop, of the appearance and consistency of glycerin, oozes from the micropyle of the ovule. As the pollen passes through the drop into the pollen chamber, at least a portion of the drop becomes brownish and so hard that it adds to the difficulty of sectioning.

THE POLLEN TUBE

Whether in artificial cultures or in the nucellus of the ovule, the pollen tube begins to grow at once, emerging from the apex of the spore and growing out into the sugar solution or into the tissues of the nucellus. The tubes are irregular in diameter and sometimes have short branches, but they are nearly straight and lie so close to the surface that their position is indicated by brown lines radiating from the beak (*figs. 19, 23*). Since the haustorial portion of the tube reaches a length of 2 or 3^{mm}, the brown lines are easily visible to the naked eye. As the tube begins to form, the pollen grain end is pushed into the pollen chamber before the haustorial end has penetrated far into the tissues of the nucellus. The tube is formed from the intine, which breaks through the exine and increases greatly in thickness, as may be seen by noting the comparative thickness of exine and intine in *figs. 8* and *18*. The difference is even greater than is indicated by the figures, because *fig. 8* is more highly magnified than *fig. 18*. The tube stains a light brown with iron alum hematoxylin, contrasting sharply with the brilliant red which the exine takes when stained with safranin.

Starch is abundant in the pollen tube, and filaments looking like the radiations about the blepharoplast, only much longer, are conspicuous, especially in the vicinity of the nucleus.

THE BODY CELL

The division of the generative cell, giving rise to a stalk cell and a body cell, takes place soon after the pollen is shed, all material collected later than the middle of October showing this division already completed.

In characteristic cycad fashion, the prothallial cell now pushes up into the stalk cell (*figs. 10, 11, 12, 15, 18*). Stages between *figs. 9* and *10*, which might show the cause of this peculiar and remarkably constant behavior of the prothallial cell, were not available.

The body cell, which is to produce two sperms, does not divide until the following spring. The division usually takes place about the first of April, but may occur a week earlier, or as late as the first week in May. During this period of about half a year, there is a gradual growth and differentiation of the body cell.

At first the cytoplasm of the body cell seems homogeneous, without any vacuoles or conspicuous granules (*fig. 10*). The chromatin and nucleoli of its nucleus stain sharply with iron alum hematoxylin. Most of the chromatin is in the form of deeply staining granules.

In a short time it is noticed that the nucleus no longer stains sharply, the reticulum appearing very faint, and even the nucleolus and larger chromatin granules taking scarcely any stain. But while these changes are taking place within the nucleus, many granules, staining sharply with iron alum hematoxylin and apparently identical with the chromatin granules, appear in the cytoplasm. For the sake of reference we may call them the *black granules* (*bg, figs. 13, 14*). I believe that they have come from the nucleus. Whether the granules pass through the membrane bodily, or become dissolved and pass through by osmosis, might be a question. Living chromatin is semi-fluid and the nuclear membrane at this time is extremely thin. If the nuclear membrane is formed by the condensation of cytoplasm about the nuclear vacuole, the "breaking-down" of the membrane in the prophase of mitosis may be merely the return of the condensed cytoplasm to the ordinary alveolar condition; and as this condition approaches, but while the membrane is still recognizable, it is reasonable to suppose that particles may pass from the nucleus to the cytoplasm without becoming soluble. The black granules might pass from the nucleus to the cytoplasm in this way. There is little doubt that chromatin is more or less soluble. In solution, the granules could pass by osmosis through a membrane with such a structure as a physiologist might imagine the nuclear membrane to have.

The black granules are very small at first and are more numerous near the nucleus. They increase in size by imbibing liquid from the surrounding cytoplasm, until the granule becomes a thin pellicle inclosing a liquid. As the pellicle stretches, granules pass through it into the watery interior, and the color with iron alum hematoxylin gradually changes from black to gray. For reference, these globules may be called *gray bodies* (*gb, figs. 14, 17*). Both the black granules and gray bodies are found not only in the body cell, but also in the stalk and prothallial cells, and even in the cells of the nucellus.

THE BLEPHAROPLAST

The origin of the blepharoplast is not easy to determine. In Zamia, Cycas, and Ginkgo, the blepharoplast, when first recognizable, is a small sharply staining granule in the cytoplasm of the body cell. We must admit that the same is true of Dioon, but a study of the black granules led us to surmise that blepharoplasts, in their origin, are simply these granules derived from the nucleus. It would follow that not two but several blepharoplasts might begin to develop. Why only two should become differentiated is not clear. The blepharoplast, in all the early stages of its development, takes an intense homogeneous black color with iron alum hematoxylin, never behaving like the gray bodies. Sometimes the cytoplasm about one or more black granules becomes dense and homogeneous, quite unlike the usual alveolar structure, and resembling the archoplasm which surrounds young centrosomes (*fig. 13*). It is possible that blepharoplasts may begin their growth in this way.

After enlarging considerably, two blepharoplasts become unmistakable through their influence upon the surrounding cytoplasm, which takes on a radiate arrangement with the blepharoplast as a center (*figs. 15, 16*). At first, the radiations are nothing more than the intersections of alveoli (*fig. 16*), but as the walls of the alveoli become less distinct, the radiations become definite granular filaments, extending from the blepharoplast to the periphery of the cell. Many of the filaments are simple, but branching is very common (*figs. 17, 22*).

The appearance of the filaments in preparations indicates a streaming movement, especially toward the blepharoplast. That the radiations seen in sections are streams of cytoplasm is indicated by the fact that exactly similar structures are found in the pollen tube, where there is certainly a strong streaming movement. An almost positive proof that the filaments are streams of cytoplasm is furnished by artifacts. In cutting out the top of the female gametophyte with its archegonia, the least pressure will cause some of the cytoplasm of the eggs to be squeezed out through the necks of the archegonia. This escaping cytoplasm, streaming out through the neck of the archegonium, shows very numerous filaments with a structure identical with that of the radiations about the blepharoplast, and in case of the artifact there is no doubt that the filaments are nothing but streams of the egg cytoplasm. In many cases the connection of the radiations with the blepharoplast also indicates a streaming (*fig. 21*).

Soon after the stages shown in *figs. 16* and *17*, the filaments appear very granular, some of the granules being almost certainly the black granules. The gray bodies become attached to the filaments and give the radiations about the blepharoplast a striking appearance (*figs. 18, 20, 21, 31*). The general topography of the pollen tubes, pollen chamber, and nucellus at this time is shown in *fig. 19*.

The watery gray body runs along the filament, usually in both directions, so that it becomes spindle-shaped, but often it spreads only in one direction, and consequently becomes top-shaped. As the gray bodies spread along the filament, depositing granular matter and giving up their watery content, the filaments become smoothly and sharply defined and have much greater density (*figs. 21, 24*). The growth of the blepharoplast is due, in great measure, to the acquisition of granules, and perhaps other matter, brought to it by the streaming filaments.

During the early stages of its growth, the body cell elongates and the two blepharoplasts with their conspicuous radiations lie in the plane of the long axis, one above and the other below the nucleus (*fig. 18*). The pollen tube, at this time, is very narrow, and this fact may account for the elongation of the body cell, which fills nearly the entire diameter of the tube.

In March the pollen tube has become very large, especially the free end of it, which projects into the pollen chamber, and with this increase in the diameter of the tube the body cell changes from an elongated to a nearly spherical form, the blepharoplasts at the same time rotating 90°, so that they become transverse to the long axis of the tube (*fig. 25*).

Even before assuming the transverse orientation, the blepharoplasts may begin to show vacuolation (*fig. 22*), but after the transverse orientation has become established, the vacuoles become so large and so numerous that they occupy nearly the entire body of the blepharoplast (*fig. 25*). They are scarcely affected by staining, in nearly all preparations showing a dirty-white or pale-yellowish color. The ground substance of the blepharoplast still continues to stain black with iron alum hematoxylin, or red with safranin.

The blepharoplast, when nearly mature, is spherical and measures 16 to 18 μ in diameter. Just before breaking up into granules, it becomes somewhat elliptical in section, with its longer axis parallel with the longitudinal axis of the body cell. The longer axis of the blepharoplast then measures about 20 μ.

THE SPERMS

The body cell divides longitudinally, giving rise to two sperm mother cells (*fig. 26a*). At about the time of this division, the blepharoplast breaks up into a large number of granules, which at first occupy the elliptical area of the blepharoplast. The granules are derived not only from the rim of the blepharoplast but from the portions between adjacent vacuoles. It is possible that granules may also be formed from the radiations, for these begin to disappear at this time. The area of granules soon becomes elongated, and the spiral band begins to appear (*figs. 26, 26a*). By this time the pollen chamber has extended until it has destroyed all that part of the nucellus lying above the archegonial chamber, so that there is no obstruction between the ends of the pollen tubes and the necks of the archegonia (cf. *figs. 19* and *23*). If the nucellus be removed, the numerous pollen tubes protruding from the pollen chamber are easily visible to the naked eye, and a little later the sperms may be observed without even the aid of a pocket lens (*figs. 27*). In this figure, the star-shaped area is a portion of the tissue of the nucellus, exposed by the rupturing of the megaspore membrane. The evenly dotted portion represents the megaspore membrane, which in this region adheres to the nucellus rather than to the female gametophyte.

As we have said, the spiral band begins to appear as soon as the area of granules elongates. The band is closely applied to the nucleus as shown in *fig. 26*. The nuclear membrane is very weak in this region, and the nuclear structure indicates a movement of material toward the point of attachment. The connection between the nucleus and the band is maintained, even after the band has come to the surface (*fig. 28*). In the mature sperm the band is a spiral of five or six turns, the direction being, almost without exception, from left to right, as viewed from above. The radiations, which were so conspicuous during the growth of the blepharoplast, disappear as the blepharoplast breaks up into granules.

The two sperms are formed within the two cells resulting from the division of the body cell. That this is the case is readily seen in sections (*figs. 28, 29, 32*). The relations are particularly clear in *fig. 29*, in which *t* is the pollen tube, and *w* the wall of the sperm mother cell. The figure shows the apex of the sperm with a small portion of its large nucleus still surrounded by the mother cell. The two sperms within their mother cells are shown in the photomicrograph, *fig. 32*.

That the sperms are formed within mother cells is also clearly seen in living material, where the mother cells enlarge considerably after the sperms are ready to move. The peripheral portion of the partition between the two sperms breaks down, thus allowing the sperms to move about within the old body cell. At this stage the term body cell is not strictly correct, because the cavity now consists of the combined areas of the two sperm mother cells. Since, however, the outer wall is still the wall of the original body cell and there is no name for the new cavity, we may refer to it as the old body cell. The wall of the old body cell soon breaks down and the sperms escape into the main portion of the tube.

Sperms within the pollen tube measure about 200 μ in diameter and about 275 μ from apex to base. After leaving the tube, they increase somewhat in size, reaching a diameter of 230 μ and a length of 300 μ. Consequently, they are easily visible to the naked eye.

The living sperm, as seen under the microscope, has a large granular nucleus, surrounded by a thin and almost colorless sheath of cytoplasm, which is somewhat thicker at the spiral end. The nucleus usually shows a large depression just beneath the apex of the sperm (*fig. 32*).

The movements of the sperms are easily observed by simply mounting a piece of the nucellus with its pollen tubes. In addition to the lighting from above, some light may be reflected up through the pollen chamber. The upturned ends of the pollen tubes (*fig. 27*) are so transparent that they scarcely obscure the view. The cilia begin to move while the sperms are still fast together and more or less attached to the stalk cell. The movement of cilia is accompanied by pulsating and amoeboid movements which continue for an hour or more before the sperms separate. After the separation, they may swim for half an hour or more in the old body cell before they escape into the general cavity of the tube. Occasionally, the sperms are still attached to each other after they have escaped into the tube, and in such cases their movements are awkward, because they naturally try to move in opposite directions. When free from each other the principal movement is straight ahead, with a rotation on the long axis. The sperms swim up and down in the tube, going up as far as the diameter of the tube will permit, and then coming back. The amoeboid movements both of the cytoplasm and the nucleus are quite noticeable, especially while the sperm is changing its direction. At the apex, where the cytoplasmic sheath is thickest, the amoeboid movement is most conspicuous, and may be so rapid that it is more like a twitching. How long the sperms might swim in the pollen tubes under natural conditions, one could hardly guess. When a nucellus is inverted in a drop of sugar solution on a slide, and is still further protected by a bell jar, the movements have continued for five hours.

After the sperms begin to move, there is a rapid increase in the turgidity of the tube, which sooner or later ruptures at or near the exine of the pollen grain. Most of the starch and liquid contents of the tube escapes with a spurt, unless one of the sperms is immediately drawn into the opening. The first sperm may escape in two or three seconds, but the other may be half a minute in getting out, probably because there is not so much pressure behind it. The rupture is often not more than 50 μ in diameter, while the average sperm is four times as broad. But however much the sperm may be constricted in getting out, it promptly regains its form and begins to swim.

Efforts to keep the sperms alive after their escape from the pollen tube were not very successful. In weak sugar solutions they immediately break to pieces, almost explode. In a 10 per cent. sugar solution they quickly die. Sugar was added to a 10 per cent. solution until it became, perhaps, a 12 or 15 per cent. solution, and in this the sperms continued to swim for several minutes. No experiments were made to determine whether the sperms are chemotactic or not.

The material would have allowed a more detailed account of the later history of the blepharoplast and the development of the sperm, but these features, particularly the relation of the blepharoplast to the spiral band, are shown so much more clearly in my preparations of Ceratozamia, that I have refrained from any extended description at this time.

The further history of the sperm of *Dioon edule* will be considered in a forthcoming paper on fertilization and embryogeny.

DISCUSSION

With THIESSEN'S (**14**) paper on the seedling of *Dioon edule*, my own paper on the ovule and female gametophyte, the present paper on spermatogenesis, and a study of fertilization and embryogeny now nearly completed, considerable information concerning this form is available. The temptation to draw conclusions is strong, but studies on *Ceratozamia mexicana* and *Dioon spinulosum* are well under way, and Sister HELEN ANGELA (**12**) has already completed an investigation of the anatomy of Ceratozamia. Since any theories will be more likely to be well founded if based upon a comparative study, I shall reserve speculations for a safer opportunity. At present I shall merely consider a few points suggested by the foregoing description.

The largest staminate sporophylls in the Cycadales are found in Cycas and the smallest in Zamia. The number of sporangia corresponds, roughly, to the size of the sporophyll. The sporangia in Cycas, Encephalartos, Dioon, and all the forms with large microsporophylls, so far as I have been able to examine them, have the sporangia in definite sori with 3, 4, or 5 sporangia in a sorus. In forms with smaller sporophylls, like Zamia, Ceratozamia, and Microcycas, some of the sporangia are single, but most of them are grouped, with 2 or 3, or occasionally 4 sporangia in a sorus. CALDWELL (**9**) states that in Microcycas the sporangia are not arranged in sori, but his photograph shows that even on the smaller sporophyll most of the sporangia are in sori; the number of sporangia is about 100 on the larger sporophyll. The number of sporangia on a sporophyll, according to Miss GRACE SMITH (**8**) is as follows: *Cycas circinalis* 700, *Encephalartos Caffer* 700, *Macrozamia Miquelii* 600, *Encephalartos villosus* 500, *Dioon edule* 200, and *Zamia floridana* 24. We can now add that the number in *Dioon edule* often reaches 300, the usual number in *Ceratozamia mexicana* is 250 to 300, and in *Stangeria paradoxa*, about 260. In both Ceratozamia and Stangeria the sporangia are very small.

If this is a reduction series, as we believe it is, Microcycas, so far as this character is concerned, has scant claim to the position of "the most primitive cycad yet described," especially since LAND (**10**) from a consideration of the female gametophyte, and Sister HELEN ANGELA (**13**) from an investigation of the vascular anatomy, have concluded that Microcycas is far from primitive. As far as microsporophylls and microsporangia are concerned, Dioon is nearer the Cycas condition than any other western cycad.

The output of spores is very easily estimated. BOWER'S studies on spore-producing members have shown that the more primitive ferns have a large output, which is gradually reduced as we pass to the highly specialized recent forms. We believe the same is true of cycads. The output in *Dioon edule* is about 30,000 spores per sporangium. According to Miss GRACE SMITH (**8**) the output in *Encephalartos villosus* is 26,000, in *Ceratozamia mexicana* 8000, and in *Zamia floridana* 500 to 600. It would be interesting to know the output in the other cycads. I should expect an output of 30,000 or more in Cycas, and should be surprised if the output in Microcycas reached 20,000. Judging from CALDWELL'S (**9**) account, I should look for an output of about 10,000 spores.

In our study of the spore coats, I looked for the third coat described by FERGUSON (**4**). The fact that only an intine and an exine are present in Dioon led me to reexamine *Pinus Laricio*. I found only an exine and an intine, as in Dioon, the third coat described by Miss FERGUSON (**4**) being merely the usual intine which her excellent technic had sharply differentiated from the exine. As preparations show and her own figures indicate, the intine, which she mistakes for a third coat, grows out to form the pollen tube.

The blepharoplast in *Dioon edule* is probably of nuclear origin. IKENO (**1**) was inclined to believe that the blepharoplasts of *Cycas revoluta* came from the nucleus, although when first recognizable they are small bodies just outside the nuclear membrane. WEBBER (**2**) finds that in Zamia the blepharoplasts originate *de novo* in the cytoplasm of the body cell, but no convincing early stages are given. CALDWELL'S (9) material was all too far advanced to show the origin of the blepharoplasts.

It seems probable that the manner in which the spiral band is formed from the blepharoplast is similar, in its main features, in all the cycads. The solid blepharoplast becomes vacuolated and then breaks up into a group of granules from which the ciliated band is formed. CALDWELL (9) describes in Microcycas a band, already distinct during the division of the body cell, and says that this band becomes broken up into fragments upon which the beginnings of cilia may be seen. His *fig. 25* indicates that the band is a section of the rim of the much vacuolated blepharoplast, while the "fragments" in his *fig. 27* are sections of the spiral band, which has already made several turns. The cilia which he figures on the inside of the fragments need confirmation.

The origin of the blepharoplast in pteridophytes has been considered by several investigators, all of whom agree that it first appears in the cytoplasm. Some find it present even from the early spermatogenous divisions, while others find it first in the cell which is to give rise to two sperm mother cells, or, occasionally, one generation earlier than this. In a very detailed account of spermatogenesis in Nephrodium, YAMANOUCHI (11) finds that two blepharoplasts first appear in the cell which is to give rise to two sperm mother cells. The blepharoplast in pteridophytes simply elongates and forms the band directly, there being no radiations, no vacuolation, nor breaking-up into a group of granules which subsequently give rise to a spiral band. While the blepharoplasts of ferns and cycads are doubtless homologous structures, no intermediate conditions have yet been found which would explain the behavior of the blepharoplasts of cycads.

In *Dioon edule*, as in nearly all gymnosperms, only two sperms are formed in the pollen tube. In a few instances I have noted four sperms in the pollen tube of *Ceratozamia mexicana*. JUEL (3) found four to twenty sperms in the pollen tube of *Cupressus Goweniana*, and naturally regarded the condition as primitive. CALDWELL (9) found sixteen or twenty sperms in *Microcycas calocoma*, and on the basis of this character claimed Microcycas to be the most primitive cycad yet described.

That the two sperms represent a reduction from a larger number is so evident that there is no need for discussion, but a word in regard to the so-called "body cell" from which they are produced may not be out of place. In the cycads, in Ginkgo, and in Coniferales the cell whose division gives rise to the "stalk cell" and body cell is often called the generative cell, probably because the stalk cell is regarded as a spermatogenous cell which has ceased to function. In Cupressus, Microcycas, and Ceratozamia all the sperms come from the body cell, the stalk cell being entirely sterile. It might be suggested that the stalk cell corresponds to the basal cell of the ordinary fern antheridium and that the dome cell corresponds to the pollen tube, while the primary spermatogenous cell corresponds to the body cell. If the dome cell, without forming the usual cover cell, should become elongated, we should have very much the same situation as that found in *Cupressus Goweniana*, *Microcycas calocoma*, and *Ceratozamia mexicana*. The diagram (*fig. 33*) indicates these homologies in an ordinary polypod fern and a cycad. The fact that in the fern the dome cell is sister to the spermatogenous cell, while in the cycad the stalk and spermatogenous cells are sisters, might be

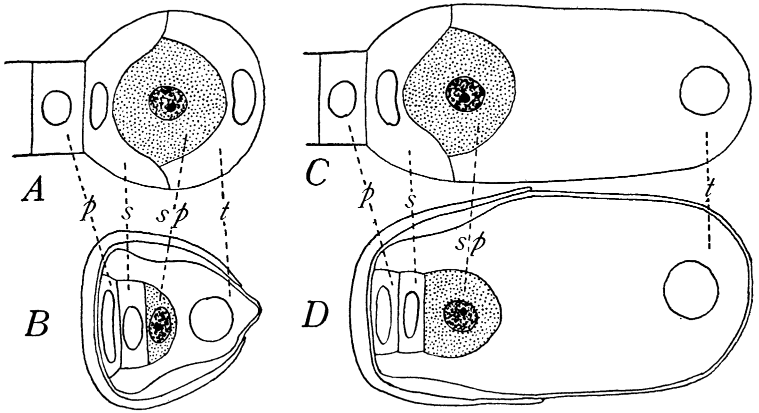

FIG. 33.—Diagram illustrating the homologies between the parts of an ordinary polypod fern antheridium and the pollen tube structures of a cycad: *A* and *C*, the fern antheridium, in *C* the dome cell being represented as elongated into a tube; *B* and *D* the cycad structures: *p*, prothallial cell; *s*, stalk cell; *sp*, spermatogenous cell; *t*, tube cell.

regarded as an objection by those who lay great stress upon the importance of a rigid sequence of cell divisions.

I have shown that in *Dioon edule* the sperms are formed within sperm mother cells from which they are subsequently discharged. IKENO (1) does not state whether the sperms of Cycas are formed within mother cells or not. WEBBER (2) claims that in Zamia they are not formed within mother cells, but that the two cells resulting from the division of the body cell become ciliated. CALDWELL (9) does not mention this feature in his account of Microcycas and his figures are noncommittal. MIYAKE (6) saw the sperms within the body cell in Ginkgo, but could not make a definite statement for Cycas. I have examined Zamia and find that the sperms are organized within definite sperm mother cells, and have found the same situation in Ceratozamia. This condition is probably general in cycads.

In bryophytes the final division of the spermatogenous cells results in conspicuous pairs of sperm mother cells, or perhaps, as has been claimed, two sperms are formed in a single mother cell. In pteridophytes the pairs are not so conspicuous, but the feature is just as definitely present. In nearly all gymnosperms the spermatogenous tissue has become reduced to a single pair of spermatogenous cells, which in some cases, as in Juniperus, bear a striking resemblance to the sperm mother cells of cycads. In Juniperus, however, no vestige of a blepharoplast has been reported, and it is assumed that the mother cells function directly as sperms. In forms like Pinus, there is merely a nuclear division within the body cell, giving rise to two sperm nuclei, no sperm mother cells being formed. In Pinus there are structures which may be vestiges of blepharoplasts. It would be interesting to know whether a cytological study of some form like Juniperus or Thuja would show non-ciliated sperms with vestiges of blepharoplasts within mother cells.

Enough is now known of the sperms of the four genera of occidental cycads to identify the genera by this character alone. A comparative study will be made later, but a few features will be mentioned here. In Cycas the spiral makes five and a half to six turns, the direction being from right to left, as viewed from above. In Zamia, according to WEBBER (2), there are five to six turns, always from left to right.

In Dioon also, the direction is from left to right. In Microcycas CALDWELL (9) figures three cases, one from right to left, and the direction of the other two uncertain. What causes the direction of spiral is not known.

Zamia has the largest sperm yet described, measuring 222 to 306 μ in diameter and 222 to 332 μ in length. In Cycas the diameter is 180 to 210 μ. In *Dioon edule* the living sperms measure about 230 μ in diameter and 300 μ in length. In microtome sections, the sperms are smaller, perhaps on account of plasmolysis, but more probably because the sperms increase in size after leaving the tube. In section the sperms measure about 200 μ in diameter and 275 μ in length. CALDWELL (9) gives no measurements, but, judging from his figures, the sperms are comparatively small, with a diameter of about 60 μ.

In all the cycads which have been studied, the movements of the sperms are very similar, a forward movement with a rotation upon the long axis. While experiments have been made, no chemotaxis has yet been noted. When the sperms are mature, the neck canal cells are very large and turgid. It may be that they exert a chemotactic influence.

If the foregoing account seems to consist rather largely of data, I can only say that I prefer to reserve any discussion of phylogeny until the investigations in which I am already engaged shall have been completed.

SUMMARY

The sporophylls of the staminate cone are rather large, and bear about 250 sporangia, a larger number than in any cycads except Cycas, Encephalartos, and Macrozamia. The output of spores per sporangium is about 30,000, a larger output than in Zamia, Ceratozamia, or Encephalartos, the only genera in which this feature has been noted.

There are twelve chromosomes in the pollen mother cell, but they often split so early that the number may appear larger.

There is only one prothallial cell and that is persistent. The report of an evanescent prothallial cell in Zamia is probably due to a misinterpretation.

The blepharoplasts are very probably of nuclear origin. The radiations are streams of cytoplasm, which, in early stages, have a peculiar appearance on account of the granules and globules which adhere to them. The solid blepharoplast becomes vacuolated and then breaks up into granules from which the spiral band is formed. The ciliated band makes five or six turns from left to right. The sperms are larger than those of Cycas or Microcycas, but not quite so large as those of Zamia.

The sperms are formed within sperm mother cells, from which they are discharged. The same is true of Zamia, Ceratozamia, and probably of other cycads.

In addition to the movement by cilia, there is a vigorous amoeboid movement of both nucleus and cytoplasm.

Discussion of phylogeny will be reserved until investigations now in progress have been completed.

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EXPLANATION OF PLATES XV–XVIII

FIGS. 1, 2.—In text.

PLATE XV

FIG. 3.—Transverse section of a young microsporophyll, showing sori, *s*, mucilage ducts, *m*, and vascular bundles, *b*. ×10.

FIG. 4.—Two microsporangia of sorus. ×55.

FIG. 5.—Section of a part of the microsporangium, showing epidermis, wall layers, tapetum, and some sporogenous tissue. ×250.

FIG. 6.—Microspore, Aug. 14, 1905. ×1260.

FIG. 7.—Microspore showing the prothallial cell, *p*, generative cell, *g*, and tube cell, *t*. ×1260.

FIG. 8.—The pollen tube is beginning to form. ×1260.

FIG. 9.—Pollen tube somewhat later. Oct. 1, 1907. ×1260.

FIG. 10.—The generative cell has divided to form the stalk cell, *s*, and body cell, *b*. Oct. 21, 1907. ×1000.

FIG. 11.—A later stage; black granules and gray bodies in the cytoplasm of the body cell. ×1000.

FIG. 12.—Nearly the same stage as *fig. 11.* ×1000.

FIG. 13.—Body cell showing black granules. Oct. 23, 1907. ×1890.

FIG. 14.—Body cell with black granules and gray bodies. Oct. 23, 1907. ×1890.

FIG. 15.—Beginning of radiations about the blepharoplasts. Oct. 30, 1907. ×945.

FIG. 16.—The blepharoplasts and radiations of the previous figures. ×1890.

PLATE XVI

FIG. 17.—Blepharoplast with radiations and gray bodies, *gb;* the figure shows the alveolar structure of the cytoplasm. ×1890.

FIG. 18.—Pollen tube structures with gray bodies on the radiations surrounding the blepharoplasts. Nov. 20, 1906. ×630.

FIG. 19.—General view of nucellus at stage shown in *fig. 18*, with pollen tubes and pollen chamber. ×30.

FIG. 20.—Blepharoplast with gray bodies on the radiations. Nov. 27, 1907. ×1890.

FIG. 21.—Later stage of blepharoplast and radiations. ×1890.

FIG. 22.—Still later stage. ×945.

FIG. 23.—View of nucellus just before the division of the body cell; the pollen chamber has extended entirely through the nucellus. ×8

PLATE XVII

FIG. 24.—Body cell with two blepharoplasts. ×945.

FIG. 25.—Pollen tube structures after the transverse orientation of the blepharoplasts. May 5, 1906. ×237.

FIG. 26.—Beginning of the spiral band. ×1890.

FIG. 26*a*.—Topography of pollen tube structures at the stage shown in *fig. 26.*

FIG. 27.—View of nucellus with pollen tubes at the stages shown in *figs. 26–29.* ×8.

FIG. 28.—Connection of the nucleus with the spiral band. ×945.

FIG. 29.—Apex of a sperm, showing that the sperm is organized within a mother cell: *t*, pollen tube; *w*, wall of sperm mother cell. ×800.

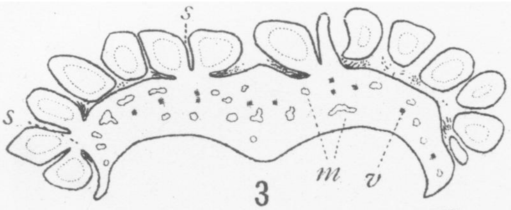
FIG. 30.—Transverse section of spiral band. ×945.

PLATE XVIII

FIG. 31.—Photomicrograph of pollen tube showing blepharoplast with gray bodies on the radiations. ×800.

FIG. 32.—Photomicrograph showing that the sperms are formed within mother cells. ×800.

FIG. 33.—In text.



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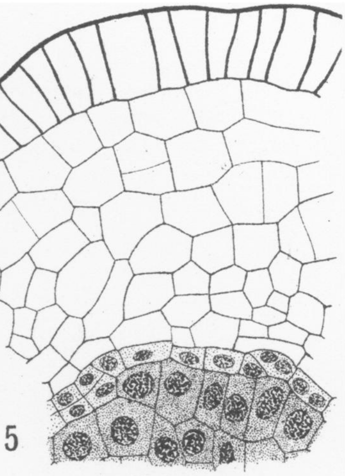

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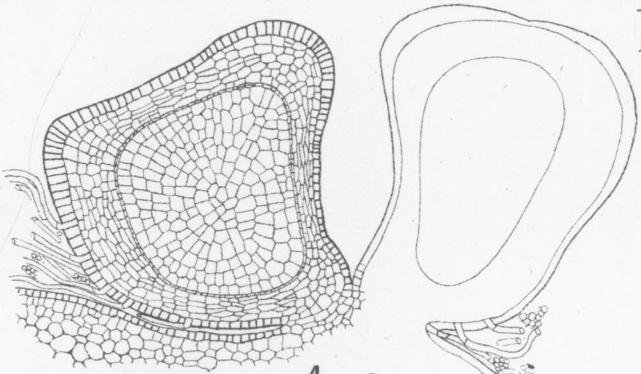

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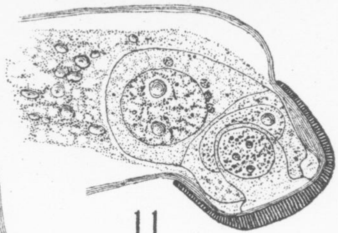

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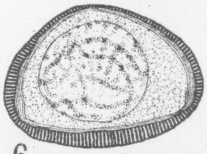

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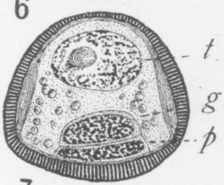

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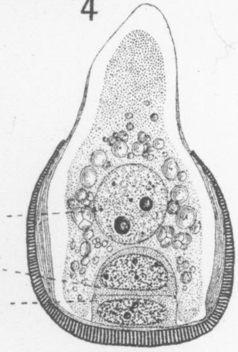

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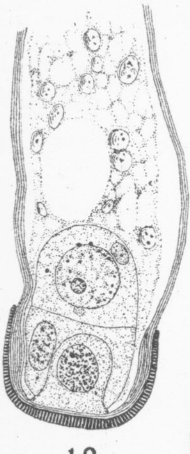

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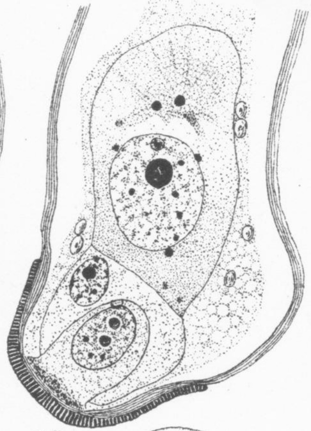

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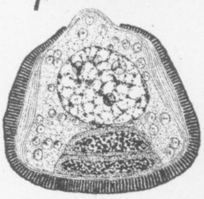

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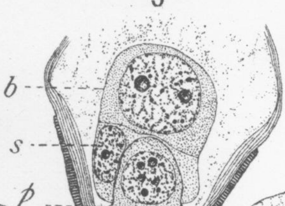

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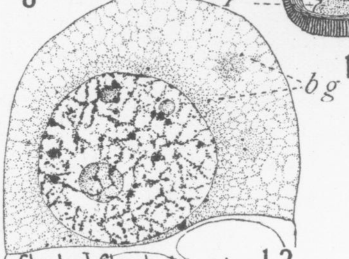

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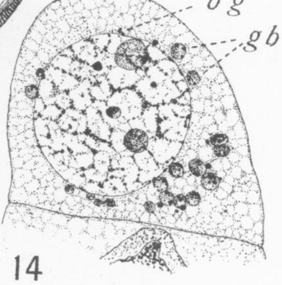

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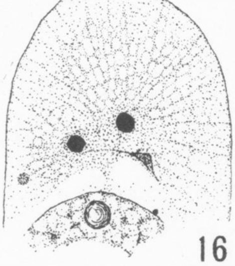

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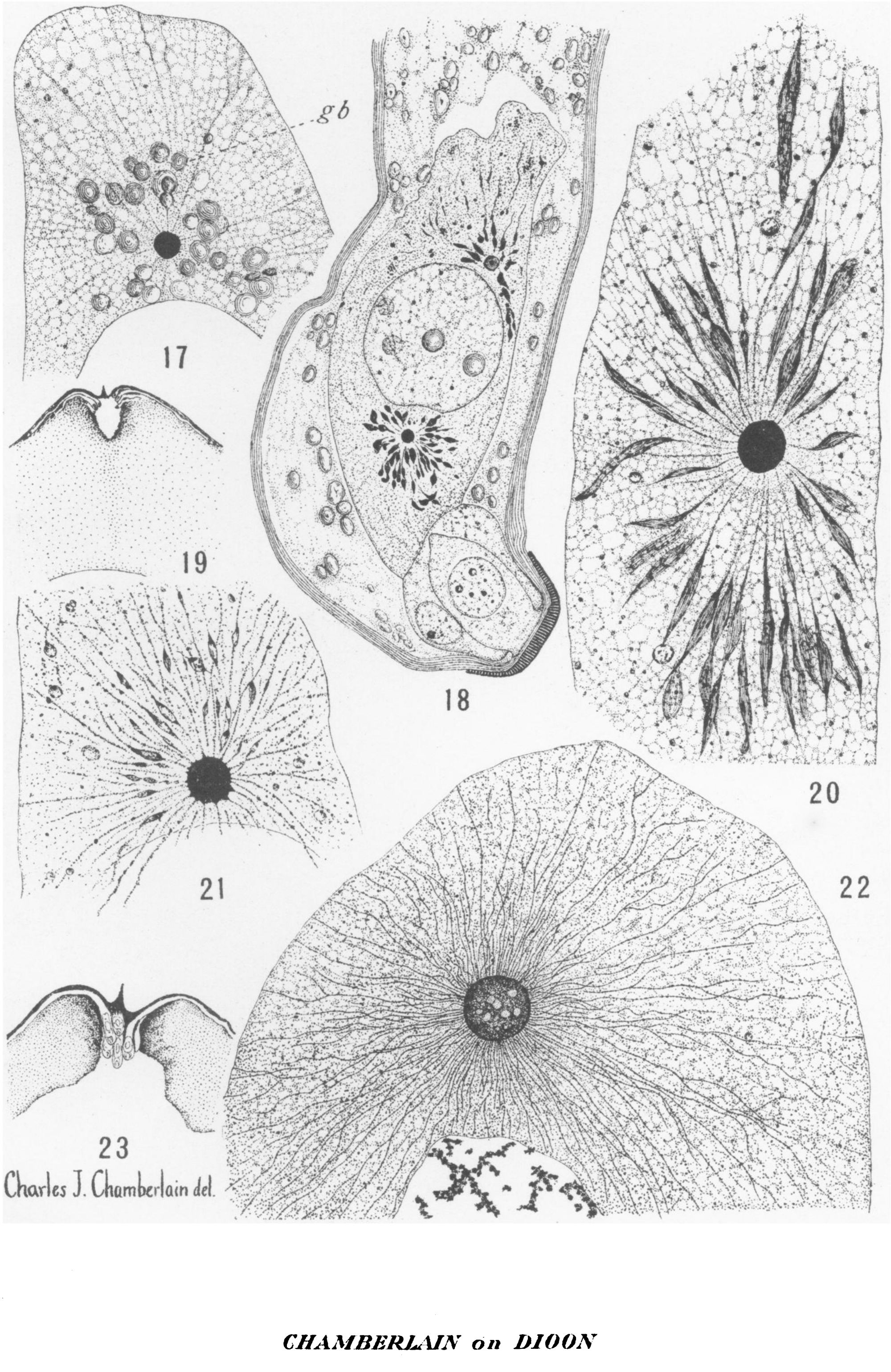

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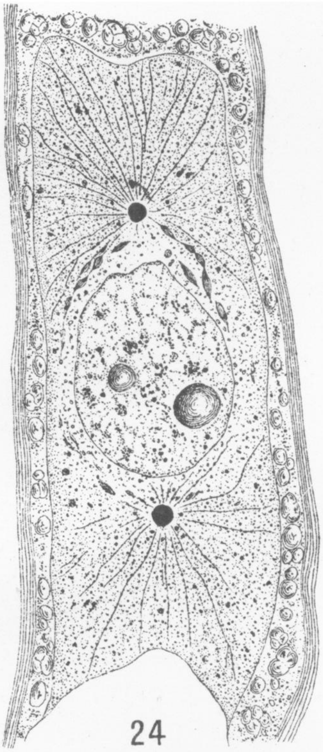

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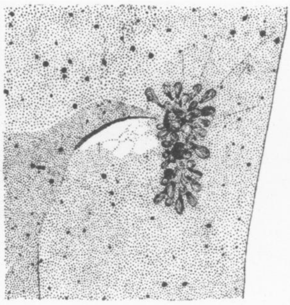

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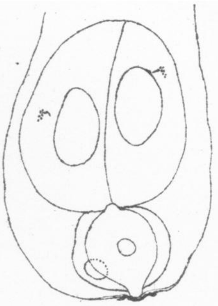

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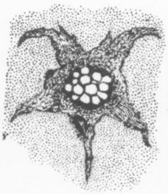

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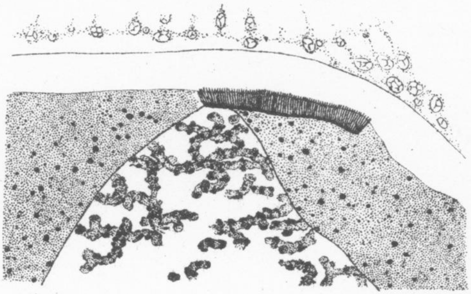

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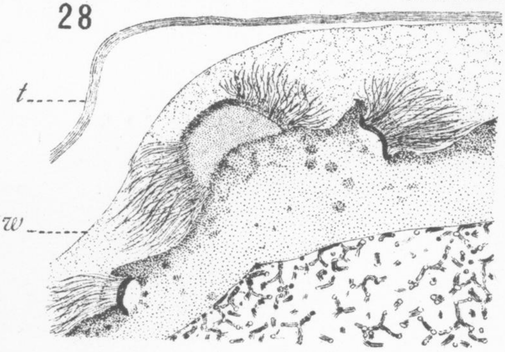

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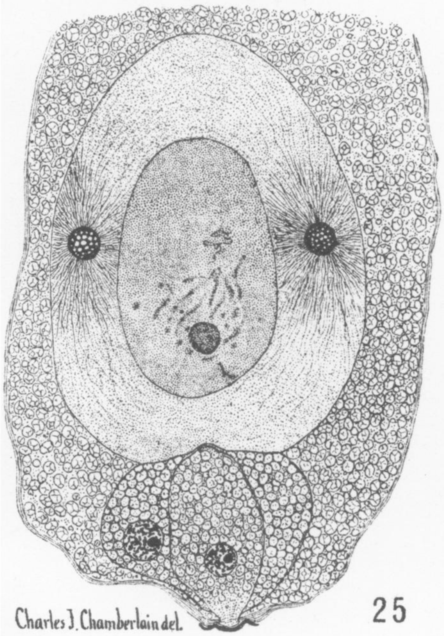

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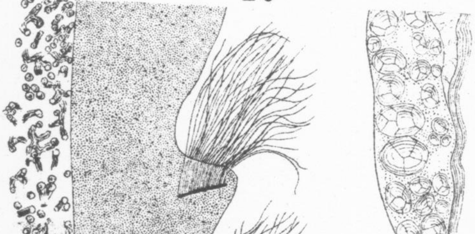

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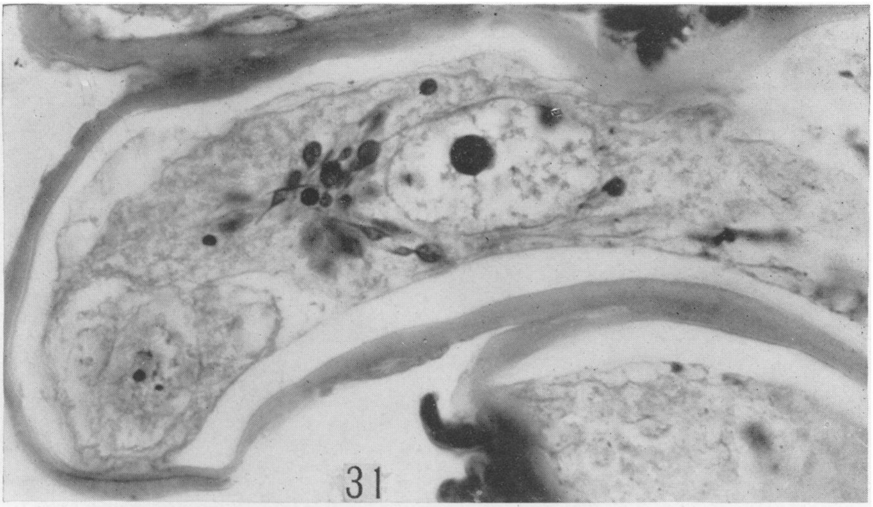
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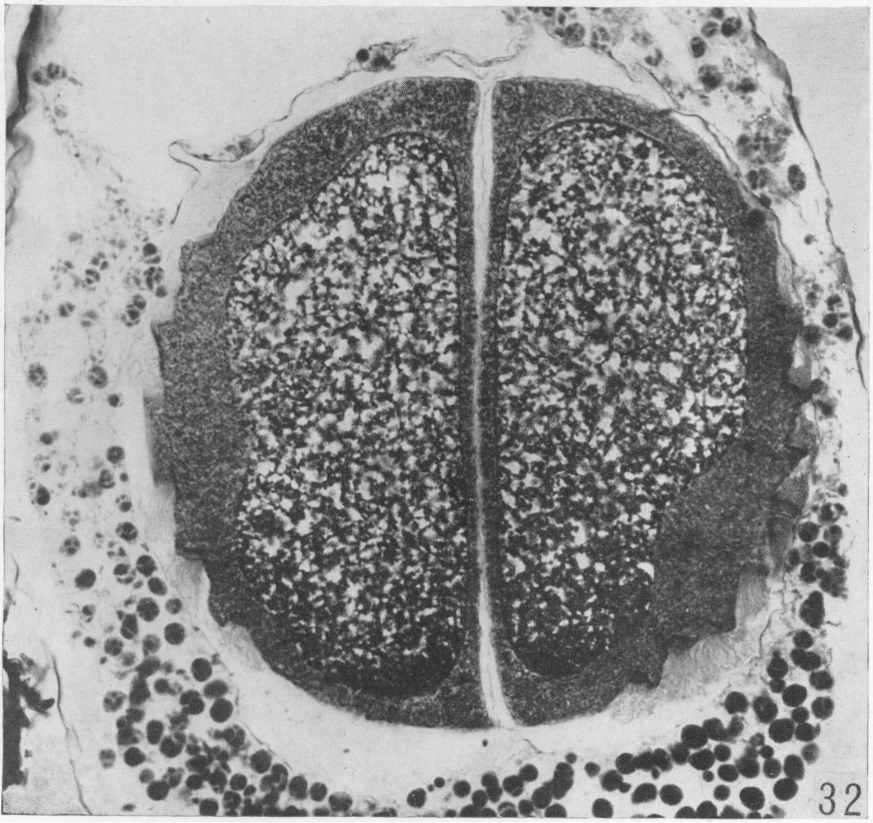
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